

XP-001038167

8

**SECRETION OF LIPOCALIN-TYPE PROSTAGLANDIN D
SYNTHASE (β -TRACE) FROM HUMAN HEART TO PLASMA
DURING CORONARY CIRCULATION**

Yoshihiro Urade,^{1,2} Yutaka Eguchi,³ Naomi Eguchi,^{2,4,5} Yoshiyuki Kijima,⁶
Yasuhiko Matsu-ura,⁶ Hiroshi Oda,⁷ Kousuke Seiki,⁷ and Osamu Hayaishi¹

¹Department of Molecular Behavioral Biology, Osaka Bioscience Institute
Suita, Osaka 565-0874, Japan

²CREST, Japan Science and Technology Corporation
c/o Osaka Bioscience Institute, Suita, Osaka 565-0874, Japan

³Intensive Care Unit, Shiga University of Medical Science
Otsu, Shiga 520-2121, Japan

⁴PRESTO, Japan Science and Technology Corporation
c/o Osaka Bioscience Institute, Suita, Osaka 565-0874, Japan

⁵Department of Morphological Brain Science, Faculty of Medicine,
Kyoto University, Kyoto 606-8315, Japan;

⁶Cardiovascular Division, Ishinkai Yao General Hospital
Yao, Osaka 581-0036, Japan;

⁷Central Research Institute, Maruha Corporation
Tsukuba, Ibaragi 300-4247, Japan

INTRODUCTION

Prostaglandin (PG) D₂ is actively formed in a variety of tissues and cells,¹ and is involved in many physiological events; e.g., it regulates sleep and ocular pressure, prevents platelet aggregation, and induces vasodilation and bronchoconstriction.^{2,3} Two distinct types of PGD synthase (PGDS), which catalyzes the isomerization of PGH₂ to PGD₂, have been isolated and characterized:⁴ one is glutathione-independent, the lipocalin-type PGDS (L-PGDS);⁵ and the other is glutathione-requiring, the hematopoietic PGDS.⁶ L-PGDS is responsible for biosynthesis of PGD₂ in the central nervous system and male genital organs of various mammals, and is secreted into the cerebrospinal fluid and seminal plasma, respectively, as " β -trace".^{7,8} In this study, we found that mRNA for human L-PGDS was most intensely expressed in the heart among various tissues examined and that the L-PGDS-like

immunoreactivity is localized in myocardial cells, atrial endocardial cells, and the synthetic state of smooth muscle cells in the arteriosclerotic plaques. We also demonstrated that the enzyme, β -trace, is secreted into the plasma of the coronary circulation of angina patients.⁹

RESULTS AND DISCUSSION

DOMINANT EXPRESSION OF L-PGDS mRNA IN HUMAN HEART

By Northern blot analysis with poly-(A)⁺RNA obtained from various human tissues (Figure 1), the mRNA for L-PGDS was detected most intensely in the heart, moderately in the brain, and very weakly in the placenta, lung, liver, skeletal muscle, kidney, and pancreas. The expression of the mRNA in human heart was much higher than that in any other tissues including the brain, whereas the expression was not detected in rat heart.¹⁰ These results indicate that the gene expression of L-PGDS is regulated in a highly species-specific manner, similar to the case of DP receptor, a prostanoid receptor for PGD₂.¹¹⁻¹³

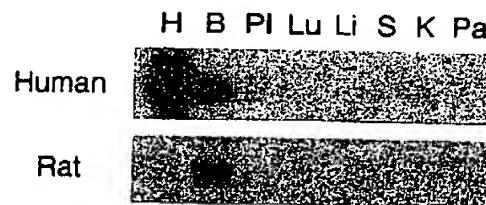


Figure 1. Tissue specificity of gene expression of L-PGDS (β -trace). Poly-(A)⁺RNA (2 μ g/lane) of various human tissues (upper lane) and total RNA (20 μ g/lane) from rat tissues (lower lane) were analyzed by Northern blot assay. H, heart; B, brain; Pl, placenta; Lu, lung; Li, liver; S, skeletal muscle; K, kidney; Pa, pancreas.

CELLULAR LOCALIZATION OF L-PGDS-LIKE IMMUNOREACTIVITY IN HUMAN HEART

The cellular localization of L-PGDS was examined by immunostaining of human autopsy specimens with monoclonal antibodies against human L-PGDS.¹⁴ Myocardial cells were positively stained, in which the immunoreactivity decreased markedly after extensive cardiopulmonary resuscitation, suggesting that L-PGDS is actively produced in beating myocardial cells and that its intracellular concentration decreases after a decrease in contraction.

The immunoreactivity was also detected in atrial endocardial cells but not in endothelial cells of the coronary artery, although both types of cells were immunoreactive with anti-von Willebrand factor antibody. In the arteriosclerotic specimens, the L-PGDS-immunoreactivity was localized in the synthetic state of smooth muscle cells in the intimal plaques and accumulated in the region of hyaline degeneration (Figure 2). However, smooth muscle cells in the contractile state were negative for L-PGDS.

Two different monoclonal and polyclonal antibodies against human L-PGDS showed essentially identical immunostaining profiles. When IgGs obtained from non-immunized animals or the polyclonal antibodies preabsorbed with excess amounts of the purified enzyme were used instead of the primary antibody, no positive immunostaining was detected.

These results suggest that L-PGDS is a useful marker for identification of the functional or differentiation stages of myocardial, endocardial, and smooth muscle cells.

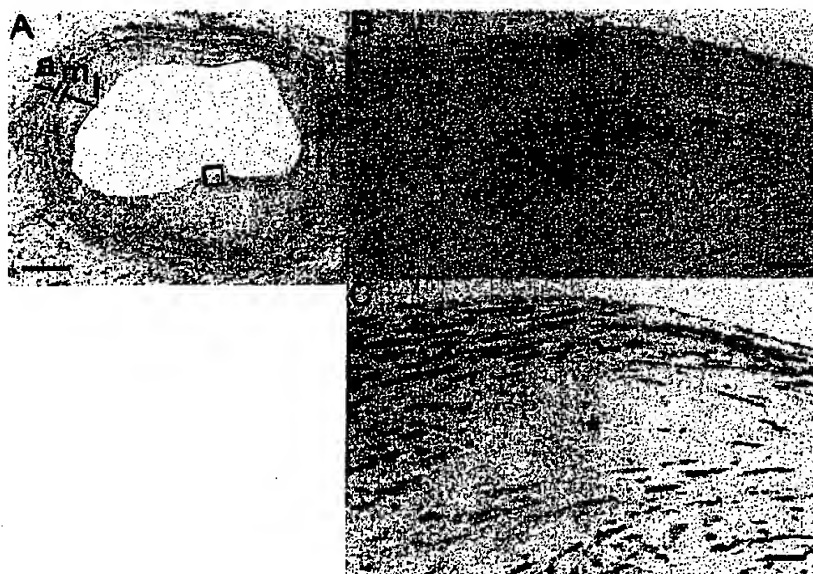


Figure 2. Immunohistochemical demonstration of L-PGDS (β -trace) in the advanced arteriosclerotic left coronary artery (50% stenosis). The serial sections were immunostained with monoclonal antibodies against L-PGDS (A, B) and alpha-smooth muscle actin (C). (B) A high-magnification view of the thickened intima (squared in A). The immunoreactivity accumulates in a region of hyaline degeneration (*). Bar = 500 μ m for A and 20 μ m for B and C. a, Adventitia; m, media; i, intima.

SECRETION OF L-PGDS INTO PLASMA DURING CORONARY CIRCULATION

L-PGDS in the brain, eye, and male genital organ is secreted into the cerebrospinal fluid,^{7,8} interphotoreceptor matrix,¹⁵ aqueous and vitreous humors,¹⁶ and seminal plasma,^{17,18} respectively. Since the enzyme may also be secreted from human heart into the plasma, we determined the plasma concentration of L-PGDS before and after coronary circulation. The plasma was collected from the orifice of the left coronary artery and great cardiac vein during coronary angiography for clinical diagnosis (Figure 3). The patients were classified into two groups, i.e., patients with stable angina and normal subjects. There were no

statistical differences between these two groups in terms of age, past history of hypertension, diabetes mellitus, habit of smoking tobacco, and serum levels of total cholesterol, triglyceride, glutamic-oxaloacetic transaminase, lactate dehydrogenase, and creatine kinase.

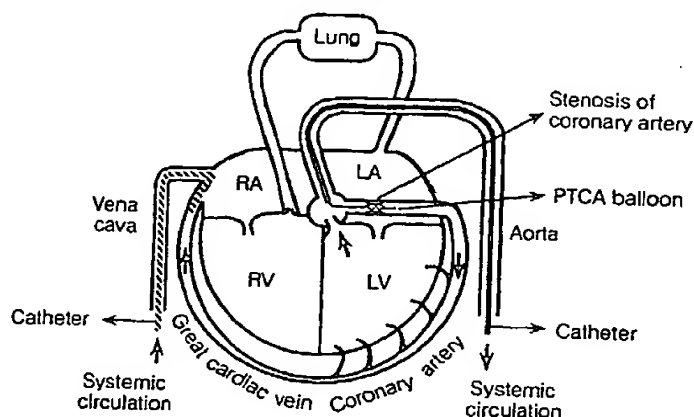


Figure 3. Schematic representation of positions of the inserted catheter used for sampling plasma.

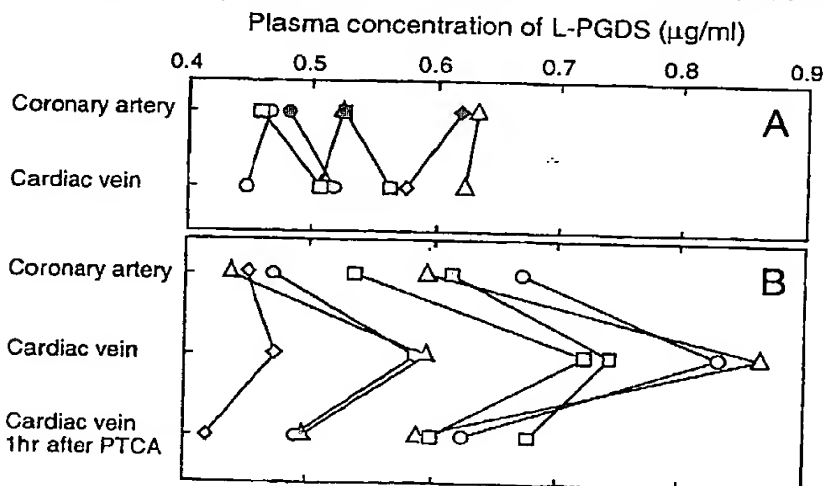


Figure 4. The plasma concentration of L-PGDS (β -trace) in the cardiac vein and coronary artery of normal subjects without stenosis (A) or of patients with stable angina (B). PTCA: percutaneous transluminal coronary angioplasty.

As shown in Figure 4, the plasma concentration of L-PGDS in the cardiac vein ($0.69 \pm 0.05 \mu\text{g/ml}$) of patients with stable angina ($n = 7$) was significantly ($p < 0.01$) higher than the concentration in the coronary artery ($0.55 \pm 0.03 \mu\text{g/ml}$), indicating that L-PGDS is accumulated in the plasma during the coronary circulation of these patients. In normal subjects without stenosis ($n = 7$), such veno-arterial difference in the plasma concentration of L-PGDS

was not observed. After percutaneous transluminal coronary angioplasty of the patients, the L-PGDS level in the cardiac vein decreased significantly ($p < 0.01$) to $0.61 \pm 0.05 \mu\text{g/ml}$ at 20 min later and reached to a level similar to the arterial level within 1 hr ($0.56 \pm 0.03 \mu\text{g/ml}$).

These results indicate that occurrence of atherosclerotic plaques of the coronary artery causes the veno-arterial difference in the plasma concentration of L-PGDS. PGD₂ may function to protect against platelet aggregation in atherosclerotic blood vessels as does PGI₂, although its antiaggregatory potency is 3- to 10-fold weaker than that of PGI₂.¹⁹

ACKNOWLEDGMENTS

This work was supported in part by grants-in-aid from the Scientific Research Program of the Ministry of Education, Science, and Culture of Japan (07558108 and 07457033), and by grants from the Sankyo Foundation of Life Science, Japan Foundation for Applied Enzymology, and The Cell Science Research Foundation. We are grateful to Drs. K. Hashimura and Y. Kato, Ishinkai Yao General Hospital, and D. Irikura, Osaka Bioscience Institute, for their skillful technical assistance.

REFERENCES

1. M. Ujihara, Y. Urade, N. Eguchi, H. Hayashi, K. Ikai, and O. Hayaishi, Prostaglandin D₂ formation and characterization of its synthetases in various tissues of adult rats, *Arch. Biochem. Biophys.* 260:521 (1988).
2. S. Ito, S. Narumiya, and O. Hayaishi, Prostaglandin D₂: a biochemical perspective, *Prostaglandins Leukotrienes Essent. Fatty Acids* 37:219 (1989).
3. M. Negishi, Y. Sugimoto, and A. Ichikawa, Prostanoid receptors and their biological actions, *Prog. Lipid Res.* 32:417 (1993).
4. Y. Urade, K. Watanabe, and O. Hayaishi, Prostaglandin D, E, and F synthases, *J. Lipid Mediator Cell Signalling* 12:257 (1995).
5. H. Toh, H. Kubodera, N. Nakajima, T. Sekiya, N. Eguchi, T. Tanaka, Y. Urade, and O. Hayaishi, Glutathione-independent prostaglandin D synthase as a lead molecule for designing new functional proteins, *Protein Engineering* 9:1067 (1996).
6. Y. Kanaoka, H. Ago, E. Inagaki, T. Nanayama, M. Miyano, R. Kikuno, Y. Fujii, N. Eguchi, H. Toh, Y. Urade, and O. Hayaishi, Cloning and crystal structure of hematopoietic prostaglandin D synthase. *Cell* 90:1085 (1997).
7. A. Hoffmann, H.S. Conradt, G. Gross, M. Nimtz, F. Lottspeich, and U. Wurster, Purification and chemical characterization of β -trace proteins from human cerebrospinal fluid; its identification as prostaglandin D synthase, *J. Neurochem.* 61:451 (1993).
8. K. Watanabe, Y. Urade, M. Mäder, C. Murphy, and O. Hayaishi, Identification of β -trace as prostaglandin D synthase, *Biochem. Biophys. Res. Commun.* 203:1110 (1994).
9. Y. Eguchi, N. Eguchi, H. Oda, K. Seiki, Y. Kijima, Y. Matsu-ura, Y. Urade, and O. Hayaishi, Expression of lipocalin-type prostaglandin D synthase (β -trace) in human heart and its accumulation in the coronary circulation of angina patients, *Proc. Natl. Acad. Sci. USA* 94:14689 (1997).
10. Y. Urade, A. Nagata, Y. Suzuki, Y. Fujii, and O. Hayaishi, Primary structure of rat brain prostaglandin D synthetase deduced from cDNA sequence, *J. Biol. Chem.* 264:1041 (1989).
11. M. Hirata, A. Kakizuka, M. Aizawa, F. Ushikubi, and S. Narumiya, Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene, *Proc. Natl. Acad. Sci. USA* 91:11192 (1994).
12. Y. Boie, N. Sawyer, D.M. Slipetz, K.M. Metters, and M. Abamovitz, Molecular cloning and characterization of the human prostanoid DP receptor, *J. Biol. Chem.* 270:18910 (1995).
13. D.Y. Gerashchenko, C.T. Beuckmann, Y. Kanaoka, N. Eguchi, W.C. Gordon, Y. Urade, N.G. Bazan, and

- O. Hayaishi, Dominant expression of rat prostanoid DP receptor mRNA in leptomeninges, inner segments of photoreceptor cells, iris epithelium, and ciliary processes. *J. Neurochem.*: in press (1998).
14. H. Oda, N. Eguchi, Y. Urade, and O. Hayaishi, Quantitative sandwich immunosorbent assay of human secretory prostaglandin D synthase (β -trace). *Proc. Japan Acad.* 72:108 (1996).
 15. C.T. Beuckmann, W.C. Gordon, Y. Kanaoka, N. Eguchi, V.L. Marcheselli, D.Y. Gerashchenko, Y. Urade, O. Hayaishi, and N.G. Bazan, Lipocalin-type prostaglandin D synthase (β -trace) is located in pigmented epithelial cells of rat retina and accumulates within interphotoreceptor matrix. *J. Neurosci.* 16:6119 (1996).
 16. D.Y. Gerashchenko, C.T. Beuckmann, V.L. Marcheselli, W.C. Gordon, Y. Kanaoka, N. Eguchi, Y. Urade, O. Hayaishi, and N.G. Bazan, Localization of lipocalin-type prostanoid D synthase (β -trace) in iris, ciliary body, and eye fluids. *Invest. Ophthalmol. Vis. Sci.* 39:198 (1998).
 17. Y. Tokugawa, I. Kunishige, Y. Kubota, K. Shimoya, T. Nobunaga, T. Kimura, F. Saji, Y. Murata, N. Eguchi, H. Oda, Y. Urade, and O. Hayaishi, Lipocalin-type prostaglandin D synthase in human male reproductive organs and seminal plasma. *Biol. Reprod.* 58:600 (1998).
 18. R.L. Gerena, D. Irikura, Y. Urade, N. Eguchi, D.A. Chapman, and G.J. Killian, Identification of a fertility-associated protein in bull seminal plasma as lipocalin-type prostaglandin D synthase. *Biol. Reprod.* 58:826 (1998).
 19. B.J.R. Whittle, S. Moncada, and J.R. Vane, Comparison of the effects of prostacyclin (PGI₂), prostaglandin E₁ and D₂ on platelet aggregation in different species. *Prostaglandins* 16:373 (1978).